Incubation Temperature and Growth of Brisbane River Turtle (*Emydura signata*) Hatchlings

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BOOTH, D.T. (1999). Incubation temperature and growth of Brisbane River turtle (Emydura signata) hatchlings. *Proceedings of the Linnean Society of New South Wales* **121**, 45–52.

Eggs of the Brisbane river turtle *Emydura signata* were incubated at three different temperatures (26°C, 28°C, 31°C) and hatchling size and growth over the first year post hatch measured in two individuals from each temperature. Hatchlings emerging from eggs at all temperatures had similar plastron lengths and head widths, but hatchling mass was significantly greater at 26°C. 412 d post-laying hatchlings from 28°C were only slightly larger in mass and dimensions than hatchlings from other temperatures, suggesting that incubation induced differences in hatchling size have minimal impact on post-hatch growth over the first year. However, incubation at higher temperature results in shorter incubation periods and early hatching may give an advantage to hatchlings when food is scare or when the immediate post hatching growth period is shortened by winter cold, causing cessation of feeding and growth.

Manuscript received 4 March 1999, accepted for publication 18 August 1999.

KEYWORDS: *Emydura*, growth, hatchling, turtle, incubation, temperature.

INTRODUCTION

Incubation temperature of many oviparous reptiles is known to affect hatchling attributes including sex (e.g. Bull 1980), morphology and size (Webb et al. 1986; Gutzke and Packard 1987; Gutzke et al. 1987; Hutton 1987; Joanen et al. 1987; Packard and Packard 1987; Packard et al. 1987; Webb et al. 1987; Webb and Cooper-Preston 1989; Burger 1990; Whitehead and Seymour 1990; Brooks et al. 1991; Demming and Ferguson 1991; Van Damme et al. 1992; Janzen 1993a; Allsteadt and Lang 1995; Shine 1995; Shine et al. 1995), behavior (Burger 1989, 1990, 1991; Webb and Cooper-Preston 1989; Janzen 1995; Shine 1995), locomotor performance (Burger 1990, 1991; Van Damme et al. 1992; Janzen 1993a, 1995; Shine 1995; Shine et al. 1995) and post-hatch growth (Hutton 1987; Joanen et al. 1987; Webb and Cooper-Preston 1989; Brooks et al. 1991; Bobyn and Brooks 1994; Rhen and Lang 1995). In species with temperature dependent sex determination, some of the differences in hatchling attributes may be due to sex, although it has been argued that this is not the case (Webb et al. 1987; Webb and Cooper-Preston 1989) and manipulative experiments that separate the effects of sex from the effects of temperature support this argument (Rhen and Lang 1995).

Temperature-induced differences in hatchling attributes are also found in species that have genotypic sex determination (Gutzke and Packard 1987; Burger 1989, 1990, 1991; Van Damme et al. 1992; Janzen 1993a; Shine 1995; Shine et al. 1995, Booth 1998). Whether or not these incubation temperature induced attributes affect a hatchling's long-term growth and/or survival is still a largely unanswered question, with few studies addressing this topic to date (Joanen et al. 1987; Webb and Cooper-Preston 1989; Brooks et al. 1991; Bobyn and Brooks 1994; Janzen 1995; Rhen and Lang 1995). Here the results of a preliminary experiment designed to quantify the effects of incubation temperature over the first year of growth in hatchling Brisbane river turtles (*Emydura signata*), a species which exhibits genotypic sex determination are presented.

MATERIALS AND METHODS

An entire clutch of 21 eggs was collected from a single female Brisbane river turtle (*Emydura signata*) nest immediately after oviposition on 24 December 1995, at a lake in St. Lucia, Queensland (27°32′S, 155°00′E). The eggs were transferred to the laboratory where they were weighed and placed in three separate containers on moist vermiculite with a water potential of approximately –150 kPa. Seven randomly chosen eggs were placed in each container, and one container placed in incubators set at temperatures of 31°C, 28°C or 26°C. Temperature within incubators fluctuated by less than 0.5°C. Towards the end of incubation, containers were examined daily for hatchlings. At hatching, each hatchling was removed, brushed free of vermiculite, weighed on an electronic balance, and plastron length and head width at the level of the jaw articulation measured with calipers. Two hatchlings from each temperature were placed into a 50 cm x 35 cm plastic tub filled to a depth of 12 cm with water. The tub was placed on a bench in front of a window in the laboratory and the water changed daily during the time when turtles were active and weekly during the winter period of inactivity. The laboratory was not artificially heated or cooled during the period of raising. Turtles were fed frozen whole mosquito fish (*Gambusia* sp.) in excess of need daily when active, and uneaten food removed when the water was changed. Food was offered once weekly during the winter period, but was never eaten. Turtles were weighed and the plastron and head width measured once a month throughout the experiment. A miniature temperature data logger (Tidbit, stowaway) programmed to record temperature every 30 min was placed in the water throughout the experiment so that mean water temperature between measurements could be calculated.

Oneway ANOVA was used to analyse for treatment differences in fresh egg mass, hatchling mass, hatchling plastron length and hatchling head width. Where significant differences were found ($P < 0.05$), a post-hoc Tukey pairwise multiple comparison procedure was used to compare individual treatments. Because sample size was limited to two turtles from each temperature, statistical analysis was not undertaken for 352 day-old turtles.

RESULTS

Of the 7 eggs incubated at each temperature, 5, 7 and 6 hatched successfully at 31°C, 28°C and 26°C respectively and subsequent analysis only includes these successfully hatched eggs. Mass and dimensions of fresh eggs, hatchlings and 412 d post-laying turtles are listed in Table 1 and the growth profiles depicted in Figure 1. Incubation time was temperature-dependent, with eggs incubated at 31°C hatching after 43 d, those incubated at 28°C after 50 d and those at 26°C after 60 d. At any one temperature all eggs hatched within 24 h of each other. Fresh egg mass and hatchling dimensions were not significantly different between temperature treatments (Table 1), but hatchlings from 26°C had a significantly greater mass than those from 31°C or 28°C (Table 1). No difference in mass was found between hatchlings from 31°C and 28°C.

Hatchlings began feeding within 4 days of hatching and grew relatively slowly (~65 mg/d) until water temperature dropped below 20°C (130 d after incubation started) at which time turtles became inactive, stopped feeding and ceased growing (Fig. 1). Approximately 4 months later (270 d after incubation started) water temperature

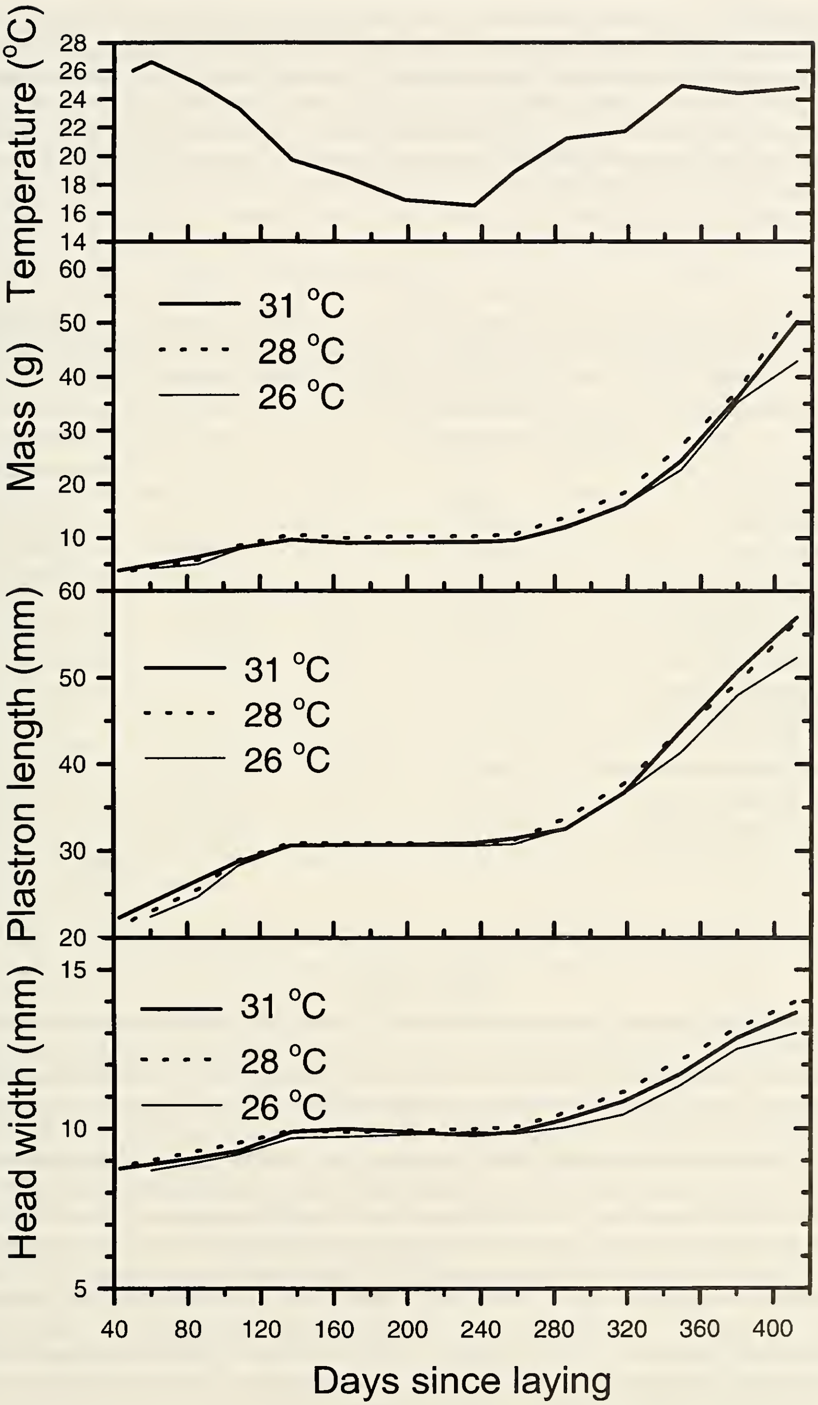

Figure 1. Mean water temperature, body mass, plastron length and head width of *Emydura signata* emerging from eggs incubated at 31°C, 28°C and 26°C against days since laying. Lines represent mean data for 2 turtles.

rose above 20°C and turtles became active and began feeding again. From this time until the experiment ceased growth was rapid (~270 mg/d; Table 1). By the end of the experiment turtles had grown to approximately 13 times their hatching mass (Table 1). Patterns of growth of turtles hatched from all temperatures were similar resulting in similar mass and dimensions 412 d post-laying (Table 1). Interestingly, growth in turtles from the 26°C treatment slowed compared to turtles from other temperatures over the last month of the experiment.

TABLE 1.

Mean mass and dimensions of fresh eggs, hatchlings and 412 d post-laying turtles and mean hatchling growth rates before and after winter of *Emydura signata* from constant temperature incubation. Numbers in parenthesis indicate sample sizes. ANOVA used for treatment comparisons. * Indicates treatment was significantly different from other treatments.

	Incubation temperature 8C			Comparison
	31	28	26	between treatments
Fresh egg mass (g)	7.100 (5)	6.882 (7)	6.998 (6)	P = 0.216
Hatchling mass (g)	3.936 (5)	3.908 (7)	4.137*(6)	P = 0.007
Hatchling plasteron length (mm)	22.4 (5)	22.2 (7)	22.3 (6)	P = 0.824
Hatchling head width (mm)	8.8 (5)	8.9 (7)	8.8 (6)	P = 0.241
412 d post-laying mass (g)	43.3 (2)	45.0 (2)	43.2 (2)	
412 d post-laying plasteron length (mm)	52.7 (2)	52.8 (2)	52.3 (2)	
412 d post-laying head width (mm)	13.2 (2)	13.8 (2)	13.0 (2)	
Pre-winter growth rate (mg/d)	56 (2)	74 (2)	66 (2)	
Post-winter growth rate (mg/d)	264 (2)	281 (2)	210 (2)	

DISCUSSION

Of the three growth parameters monitored during this experiment, body mass was chosen because it is the best overall indicator of body size, plastron length was chosen because it is the most reliable linear measurement of body size in hatchlings of this species (the carapace remains relative soft and still retains curled carapace edges for up to a week after hatching), and head width was chosen because it reflects bite size which may be important in determining what food is available to hatchlings. Hatchlings emerging from eggs incubated at 26°C were significantly heavier than hatchlings from 31°C or 28°C, but linear dimensions were similar across all three temperatures. This observation is consistent with previous studies which indicate incubation at lower temperatures results in longer incubation times and slightly larger hatchlings, as judged by either a length dimension, mass or both (Webb et al. 1986; Gutzke and Packard 1987; Gutzke et al. 1987; Hutton 1987; Joanen et al. 1987; Webb et al. 1987; Packard and Packard 1987; Whitehead and Seymour 1990; Brooks et al. 1991; Shine 1995). An exception to this generalization is the Smooth soft shell turtle (*Apalone mutica*) in which cooler incubation temperatures result in smaller hatchlings (Janzen 1993a).

Conventional wisdom suggests that bigger is better in survival stakes (e.g. Brockelman 1975; Janzen 1993b, 1997), so *E. signata* hatchlings from 26°C may have a greater fitness compared to hatchlings from 31°C or 28°C. However, in snapping turtles (*Chelydra serpentina*), incubation temperature-induced larger hatchlings do not experience greater post-hatch growth or survival compared to their smaller siblings under laboratory conditions (Brooks et al. 1991; Bobyn and Brooks 1994), similar to those in the current study. On the other hand, the incubation period at 26°C was longer than eggs incubated at 28°C or 31°C (7 and 17 days respectively). If there is an advantage to hatching and entering the aquatic environment earlier (e.g. starting to feed earlier) then hatchlings from 26°C might be at a disadvantage, despite their larger hatchling mass. Indeed, a close examination of the growth curves based on the time since laying (Fig. 1) indicate hatchlings from 26°C have similar mass but slightly smaller plastron and head width dimensions compared to hatchlings from 31°C and 28°C which have been in water and feeding for several days by the time 26°C hatchlings emerged. However the larger hatching size and slightly faster mean daily growth rate of hatchlings from 26°C compared to hatchlings from 31°C (Table 1) before the onset of winter inactivity, meant that turtles from these two temperatures were the same size at the onset of winter (Fig. 1) despite hatchlings from 26°C hatching 17 days later than hatchlings from 31°C. The faster growth rate of hatchlings from 28°C meant that they were slightly larger in mass than hatchlings from 26°C and 31°C at the onset of winter (Fig.1). After winter hatchlings from 26°C grew slower than hatchlings from 28°C and 31°C, particularly during the last month of the experiment (Fig.1, Table 1). Hatchlings from 28°C may have had an advantage over hatchlings from other temperatures because their growth rate was slightly faster both before and after winter (Table 1) which resulted in these turtles being slightly larger at the end of the experiment (Fig.1, Table 1). Comparative data on the effects of incubation temperature on the post-hatch growth of reptiles are scarce and all of the studies have raised hatchlings under highly artificial conditions. In the Nile crocodile (*Crocodylus niloticus*) individuals hatching from eggs incubated at 34°C were smaller in length, than individuals hatching from eggs incubated at 31°C, but by three months individuals from 34°C eggs were slightly longer (Hutton 1987). In American alligators (*A. mississippiensis*) hatchlings emerging from eggs at high (32.8°C) and low (29.4°C) are larger than at an intermediate temperature (30.6°C), but after 18 months of growth, the hatchlings from the intermediate temperature were significantly larger than those from either 32.8°C or 29.4°C (Joanen et al. 1987). In snapping turtles (*C. serpentina*) hatchlings emerging from eggs incubated at 28.6°C were smaller compared to hatchlings emerging from eggs incubated at either 22.0°C or 25.6°C, but once again hatchlings from the intermediate temperature were larger than either of the other temperatures after 7 months (Brooks et al. 1991) and 11 months growth (Bobyn and Brooks 1994). In another experiment with *C. serpentina*, in which temperature effects were experimentally separated from sex effects by manipulating embryo sex via the application of sex hormones during development, hatchlings from an intermediate temperature of 26.5°C grew faster than hatchlings from either 24°C or 29°C during the first 6 months of post-hatching growth (Rhen and Lang 1995). Thus the general trend from all of the studies is for hatchlings emerging from intermediate temperatures to grow faster than hatchlings from either higher of lower temperatures, but this difference in growth is small. It is highly questionable whether trends from an artificial environment (no predators, excess food at all times) can be extrapolated to the natural environment, but if there is an advantage to hatchlings emerging from eggs incubated at a particular temperature, it is slight in terms of post-hatch growth performance. If in the natural environment food is scarce and therefore a potentially limiting factor on growth, then it is probably advantageous to hatch earlier rather than later so that the limited food resources can be exploited before hatchlings from other nests emerge. In such cases, hatchlings emerging from eggs incubated at higher temperatures would have a clear advantage over hatchlings emerging from eggs incubated at lower temperatures because they hatch earlier. Hatching earlier may also be advantageous because early hatchlings have a longer feeding period before the on set of cool winter temperatures cause a cessation of feeding activity.

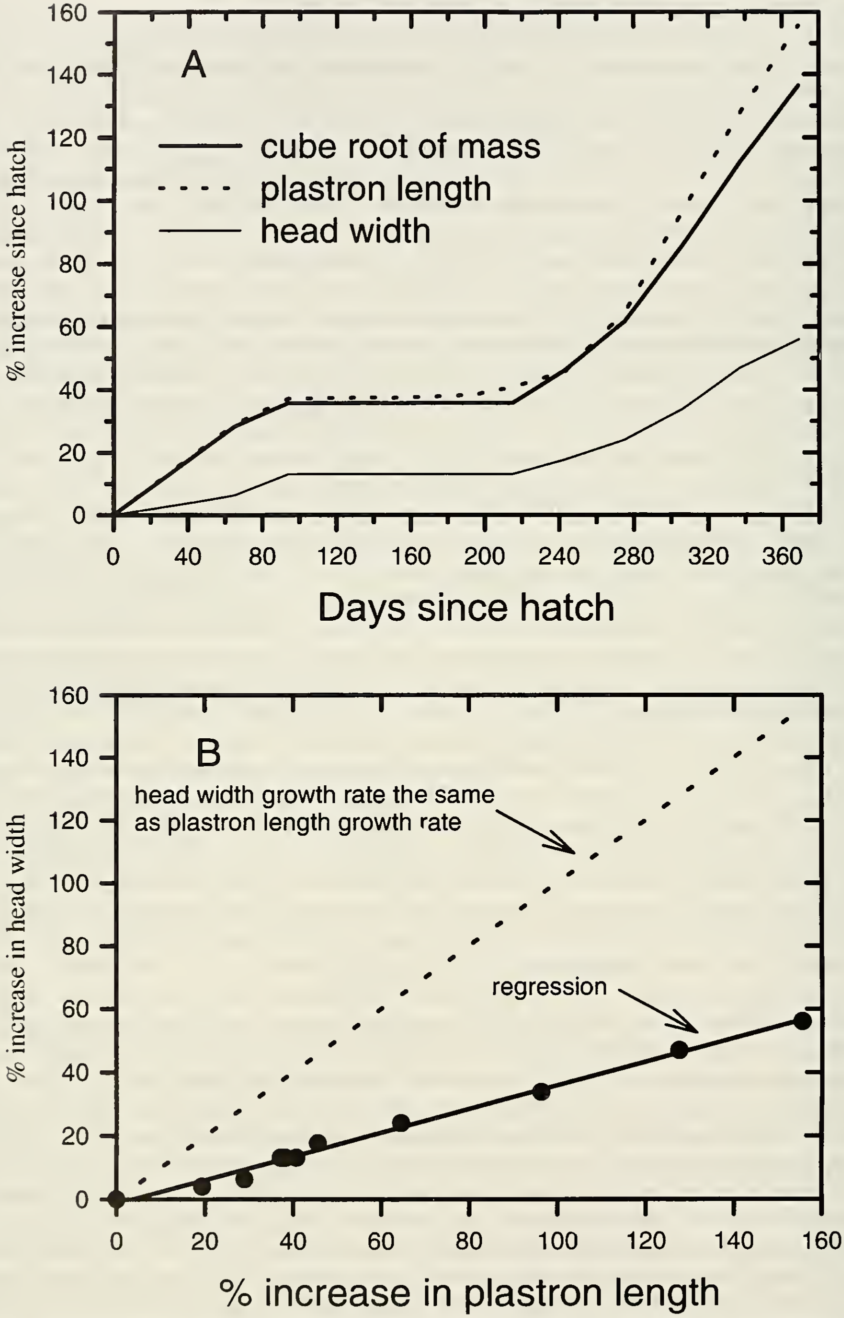

Figure 2. A). Percentage increase in cube root of body mass, plastron length and head width in *Emydura signata* hatchlings emerging from eggs incubated at 31°C. Lines represent mean data for 2 turtles. B). Percentage increase in head width against percentage increase in plastron length in *Emydura signata* hatchlings emerging from eggs incubated at 31°C. Dashed line indicates relationship if head width growth rate was the same as plastron growth rate. Solid line indicates linear regression of actual data. The slope of the regression line is considerable less than the slope of the equal growth rate line indicating that head width growth is proportionally slower than plastron length growth.

Head width reflects jaw width which in turn reflects mouth size and presumably feeding ability. If feeding ability is of particular importance to small hatchlings, the rate of head growth might be expected to be different from that of the overall body (other factors such as rate of brain growth may also have an important influence on the pattern of head growth). A plot of the relative changes in body size parameters as growth proceeds should highlight any differences. Because body mass is approximately proportional to body volume, and volume is proportional to the cube of a body linear dimension, the best direct comparison is to compare the cube root of mass with linear dimensions. A plot of these variables in hatchlings that emerged from eggs incubated at 31°C reveals that the cube root of mass scalar and plastron length have a very similar pattern of increase (as expected), but that head width increases at a much slower rate (Fig. 2A). A plot of the proportional increase in head width against the proportional increase in plastron length (Fig. 2B) also indicates head growth increases at a slower rate than body growth. Similar plots for hatchlings that emerged from eggs incubated at 28°C and 26°C show the same pattern (not shown). These plots indicate that hatchlings have proportionally much larger heads and mouths than older individuals. As a consequence, hatchling have the potential to handle relatively large food items which should enable them to tackle a wider size range of food items and thus increase their chance of survival.

ACKNOWLEDGMENTS

I thank C. Manolis and G. Webb and an anonymous referee for helpful comments on an earlier draft of this paper. This experiment was approved by the University of Queensland animal ethics committee. *E. signata* eggs were collected under Queensland National Parks and Wildlife Service permit number E4/000091/95/SAB.

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